Chemistry & Biology
Previews

Coq6 Hydroxylase: Unmasked and Bypassed

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DOI 10.1016/j.chembiol.2011.09.006

Coenzyme Q is a polyisoprenylated benzoquinone lipid essential in cellular energy metabolism. Ozeir et al. (2011) show that an enzyme, Coq6, is required for the coenzyme Q C5-ring hydroxylation, and that defects in Coq6 can be bypassed by providing alternate ring precursors.

Coenzyme Q (ubiquinone or Q) is an essential lipid quinone in respiratory electron transport. Q accepts electrons and protons from complex I and complex II, and the reduced hydroquinone (QH_2) is oxidized by complex III. Q also serves as an essential redox cofactor in the oxidation of fatty acids, glycerol-3-phosphate, dihydroorotate, sulfide, choline, and other amino acid-derived metabolites. QH_2 functions as a lipid soluble chain-breaking antioxidant, and also functions as a coantioxidant maintaining vitamin E in its reduced state (Turunen, et al., 2004). Yet unlike vitamin E, QH_2 is synthesized de novo by human and other animal cells.

Studies with the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have identified many of the genes required for Q biosynthesis (Kawamukai, 2009; Tran and Clarke, 2007). However, the functional roles of several of the *COQ* gene products (including Coq4, Coq6, and Coq9) remain mysterious. Previous work identified the *COQ6* gene as encoding a flavin-dependent hydroxylase required for synthesis of Q

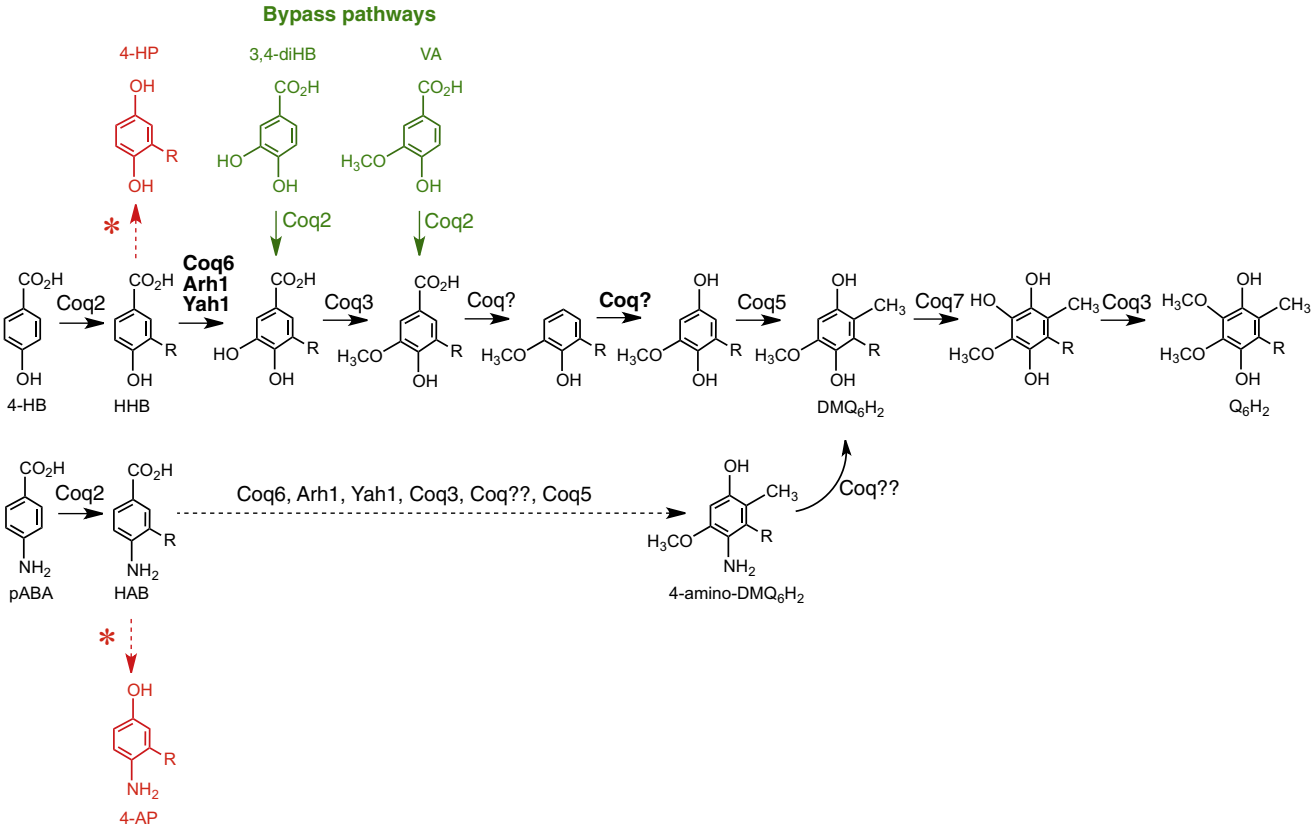

Figure 1. Many Paths to Q in Yeast
S. cerevisiae utilize 4-hydroxybenzoic acid (4-HB) and para-aminobenzoic acid (pABA) as ring precursors in the synthesis of Q_6H_2 (Marbois et al., 2010; Pierrel et al., 2010). Coq2 attaches the polyisoprenyl tail (designated as R; in *S. cerevisiae* hexaprenyl-diphosphate), generating 3-hexaprenyl-4-hydroxybenzoic acid (HHB) and 3-hexaprenyl-4-aminobenzoic acid (HAB). The 4-HB and pABA pathways are speculated to converge at the point of 4-amino-DMQ_6H_2 to demethoxy-Q_6H_2 (DMQ_6H_2) (Marbois et al., 2010). The studies of Ozeir et al (2011) show that 3-hexaprenyl-4-aminophenol (4-AP) and 3-hexaprenyl-4-hydroxyphenol (4-HP) accumulate in yeast *coq6* and *yah1* mutants fed pABA and 4-HB, respectively (compounds denoted by red asterisks). The *coq6* or *yah1* defect in Q biosynthesis can be bypassed by feeding the alternate ring precursors, 3,4-dihydroxybenzoic acid (3,4-diHB) or vanillic acid (VA) (compounds denoted in green).

in yeast (Tran and Clarke, 2007). Although the yeast *coq6* null mutant accumulated 3-hexaprenyl-4-hydroxybenzoic acid (HHB) (Figure 1), this same early intermediate was found to accumulate in each of the *coq* null mutants (*coq3*–*coq9*), and thus was not diagnostic of the blocked step (Tran and Clarke, 2007). Many of the yeast *coq* null mutants (including the *coq6* null mutant) were shown to lack other Coq polypeptide partner proteins, due to the destabilization of a large Coq polypeptide complex required for Q biosynthesis.

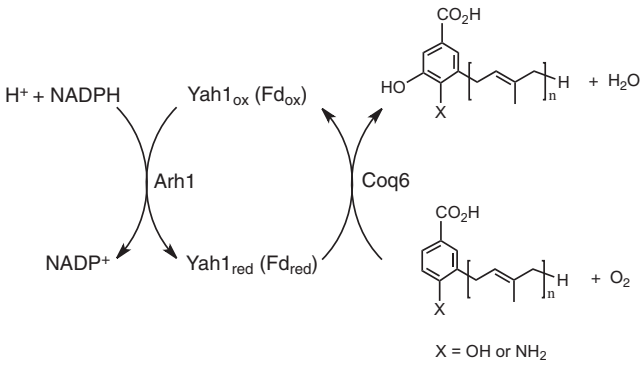

Figure 2. The *S. cerevisiae* Coq6 Monooxygenase Requires an Additional Electron Transport System
Coq6 is shown to work in conjunction with Yah1 (ferredoxin) and Arh1 (ferredoxin reductase).

In this issue, Ozeir et al. (2011) identify the function of the Coq6 polypeptide as required for the C5-hydroxylation in yeast coenzyme Q biosynthesis. The authors use a combination of yeast genetics, lipid biochemistry, and clever feeding experiments of alternate ring precursors to characterize the defective step in yeast *coq6* mutants. The authors capitalize on the observations that several of the yeast *coq* null mutants harboring multicopy *COQ8* have restored steady state levels of the Coq3 and Coq4 polypeptides (Zampol, et al., 2010), and, in the case of the *coq7* null, accumulate DMQ_6, an intermediate just two steps removed from Q (Padilla, et al., 2009). Ozeir et al. (2011) show: (1) the yeast *coq6* null mutants harboring the *COQ8* gene on a multicopy plasmid produce intermediates lacking the C5-ring hydroxyl group; and (2) yeast *coq6* null mutants expressing inactive Coq6p with two amino acid substitution mutations (Coq6-G386A,N388D) also accumulate the same two intermediates lacking the C5-ring hydroxyl, namely, 3-hexaprenyl-4-aminophenol (4-AP) and 3-hexaprenyl-4-hydroxyphenol (4-HP) (Figure 1). These intermediates are shown to accumulate when the yeast are supplied with either para-aminobenzoic acid (pABA) or 4-hydroxybenzoic acid (4-HB) as the respective aromatic ring precursors.

The authors identified 4-AP and 4-HP previously, and showed that they accumulate in two other yeast mutants deficient in either ferredoxin (Yah1) or ferredoxin reductase (Arh1) (Pierrel et al., 2010). In the present study, the *coq6*-, *yah1*-, *arh1*-, and *flx1* (*FLX1* encodes a mitochondrial FAD transporter)- deficient yeast mutants are connected by their identical accumulation of 4-AP and 4-HB. Thus, unlike most flavin-dependent monooxygenases, the electrons from NADPH are funneled indirectly to yeast Coq6 via ferredoxin reductase and ferredoxin (Figure 2).

The tour de force of the study are the bypass feeding experiments, where alternate aromatic ring precursors are supplied that rescue the *yah1* deficient mutant and the *coq6* null mutant harboring multicopy *COQ8*. These precursors include 3,4-dihydroxy-benzoic acid (3,4-diHB) and vanillic acid (VA) (Figure 1). By supplying either of these precursors, the authors demonstrate that the *coq6* and *yah1* deficient yeast mutants now acquire the ability to grow on nonfermentable carbon source and synthesize Q_6. These bypass experiments convincingly demonstrate that the defect in Q biosynthesis in the *coq6* yeast mutant is due to the lack of C5-ring hydroxylation.

A recent report identified Q deficiencies in patients with mutations in the human homolog of Coq6 (Heeringa et al., 2011). Expression of the human Coq6 polypeptide in yeast *coq6* null mutants was shown to rescue growth on nonfermentable carbon source and to restore synthesis of Q_6. Together with the findings of Ozeir et al. (2011), these results indicate that both human and yeast Coq6p function at the C5-ring hydroxylation step. In a prescient review discussing potential alternate pathways of coenzyme Q biosynthesis, Olson and Rudney (1983) noted that 3,4-diHB and VA could supply ring precursors for prenylation via metabolism of tyrosine and norepinephrine. Now, as suggested by Ozeir et al. (2011), it is possible that the deficiency in Q biosynthesis in certain human patients could be corrected by administering such alternate ring precursors.

The use of pABA by human or animal cells as a metabolic precursor in Q biosynthesis is possible, but so far has not been shown experimentally. It will be interesting to determine whether the Coq6-defective patient-derived cells or cell lines accumulate 4-AP or 4-HP as Q intermediates. It is not yet known whether 4-AP and/or 4-HP are productive Q intermediates. If so, then not only are there multiple pathways to Q, but these pathways may be branched, with flexibility in the metabolic sequence of steps similar to that of bile acid synthesis.

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